EVOLUTIONARY RELATIONSHIPS BETWEEN THE ASIAN AND NORTH AMERICAN *TRILLIUM* SPECIES

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Plants of the genus *Trillium* in Trilliaceae have a disjunct distribution, occurring in both Asia and North America, but not in Europe. In North America a total of 36 diploid species of the pedicellate and sessile flowered species grow; in contrast, in Asia only 11 pedicellate and polyploid species, including a single diploid, occur (FUKUDA 1968, 1973, 1989; SAMEJIMA and SAMEJIMA 1987).

Why have different patterns of speciation developed in North America and in eastern Asia? Which has the original site located, and how did plants migrate from that original place across the Pacific Ocean?

Since 1956 the author has undertaken a series of population analyses by means of chromosome banding analysis (DARLINGTON and La COUR 1940; FUKUDA 1962, 1967, 1969, 1970, 1984, 1987, 1988, 1989; FUKUDA and CHANNELL 1975; FUKUDA and GRANT 1979, 1980; FUKUDA and KOZUKA 1958; KURABAYASHI 1960; STEBBINS 1971).

Recently the use of isozyme analysis by the electrophoresis has proven useful in clarifying the evolutionary relationship of a polyploid level (ROOSE and GOTTLIEB 1976).

This paper will analyze the evolutionary relationship between the Asian and North American *Trillium* species by means of electrophoretic analysis and will discuss the above evolutionary questions on the basis of accumulated chromosome data.

Materials and Methods

a. *Collected Materials*

Sample plants of *T. kamtschaticum*, *T. tschonokii*, and *T. apetalon* from Japan, and *T. erectum* from U.S.A., *T. grandiflorum* from Canada were collected as is shown in Table 1. These collected rhizomes were transplanted in the *Trillium* garden at Tokyo Woman's Christian

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Table 1. Locality data for the collected samples.

Species	Locality
Trillium grandiflorum	Ste. Anne de Bellevue, Quebec, Canada
Trillium erectum	Akron, Ohio, U.S.A.
Trillium kamtschaticum	Akkeshi, Kushiro, Hokkaido, Japan
Trillium tschonoskii	Kawayu, Kushiro, Hokkaido, Japan
Trillium apetalon	Hakodateyama, Oshima, Hokkaido, Japan

University. Fresh materials were prepared there for the electrophoretic experiments.

b. *Experiments of the Electrophoresis*

Fresh leaf blades (100 mg) as the sample materials taken from each individual and smashed with the extract solution (potassium phosphate buffer, 0.05 M, pH 7.0). After centrifugation by disposable microcentrifuge tube, crude extracts of the sample were put in thin-layer acrylamide gel. Thin-layer acrylamide slab gel ($1 \times 140 \times 140$ mm) was used for experiments of the electrophoresis. Thirteen colums ($1 \times 4 \times 15$ mm) were prepared and the extrat samples were placed in the colums by micropipet. The electrode solution was 0.02 M HCl and 0.02 M ethylenediamin. The electrical conditions were 20 mA for 180 minutes.

c. *Assay*

The dye solution for the staining of the Esterase enzyme has used the fast blue RR salt by dissolving in $Na_2HPO_4 \cdot 12\ H_2O$ KH_2PO_4 solution (1/15 M phosphate buffer, pH 7.0). The substrate buffer was a mixture of α-naphthyle acetate and aceton. The gel was soaked one minute in the staining buffer before placing in the substrate buffer. Peroxidase activity was stopped by 7% acetic acid.

Results

Fig. 1 shows the findings on the peroxidase bands of the Esterase enzyme for the five Asian and American *Trillium* examined. Three or four isozyme bands appeared in the anodal electrophoretic zones between 35 mm and 50 mm from the starting points. These peroxidae bands were named a, b, c and d as is illustrated in Fig. 2. The a, b and d bands existed in all the five species, the Asian *T. apetalon*, *T. tschonoskii* and *T. kamtschaticum*, and the American *T. erectum* and *T. grandiflorum*, but the c band appeared only in *T. grandiflorum*. Although the experiments were repeated several time, the results were always the same.

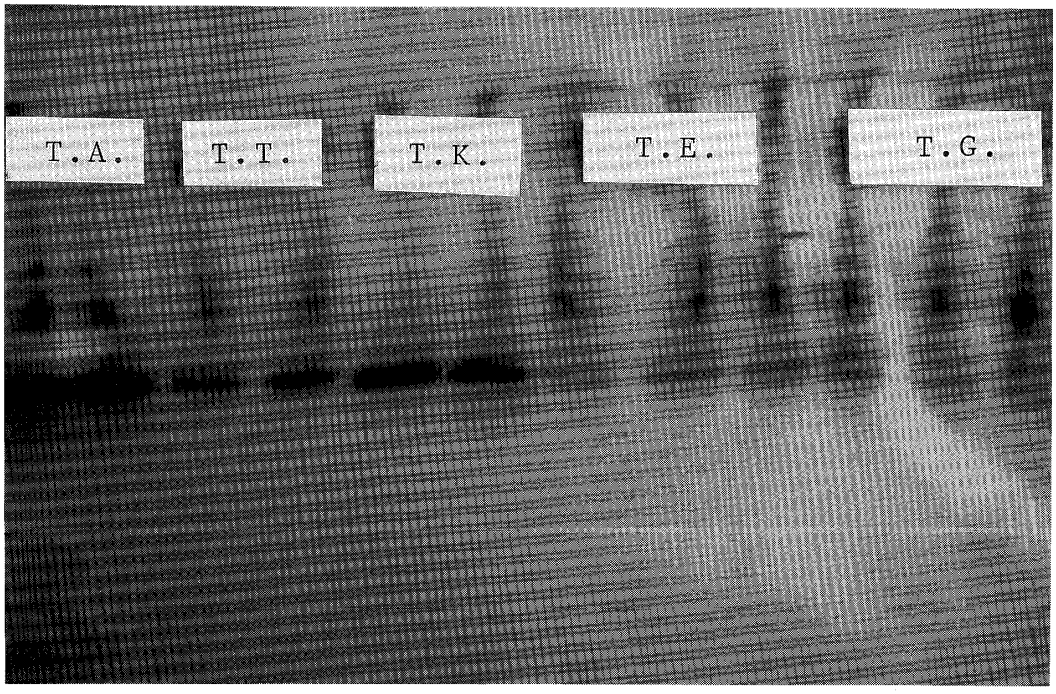


Fig. 1. Isozyme banding phenotypes of Esterase in the *Trillium* species. TA: *T. apetalon*, TT: *T. tshonoskii*, TK: *T. kamtschaticum*, TE: *T. erectum* and TG: *T. grandiflorum*.

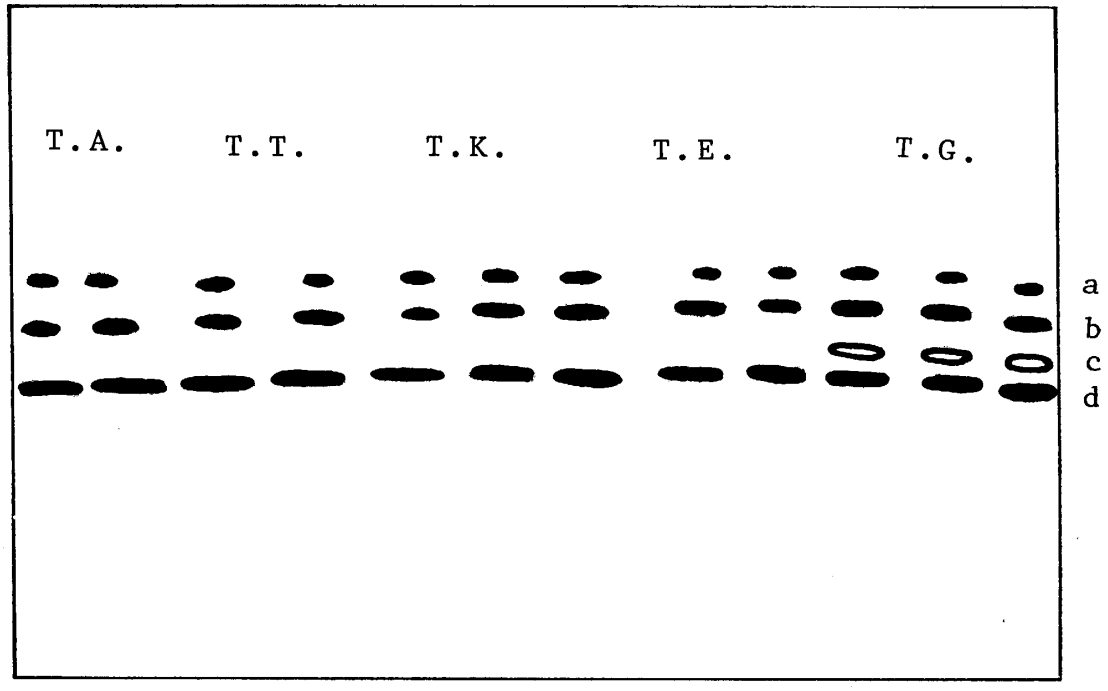
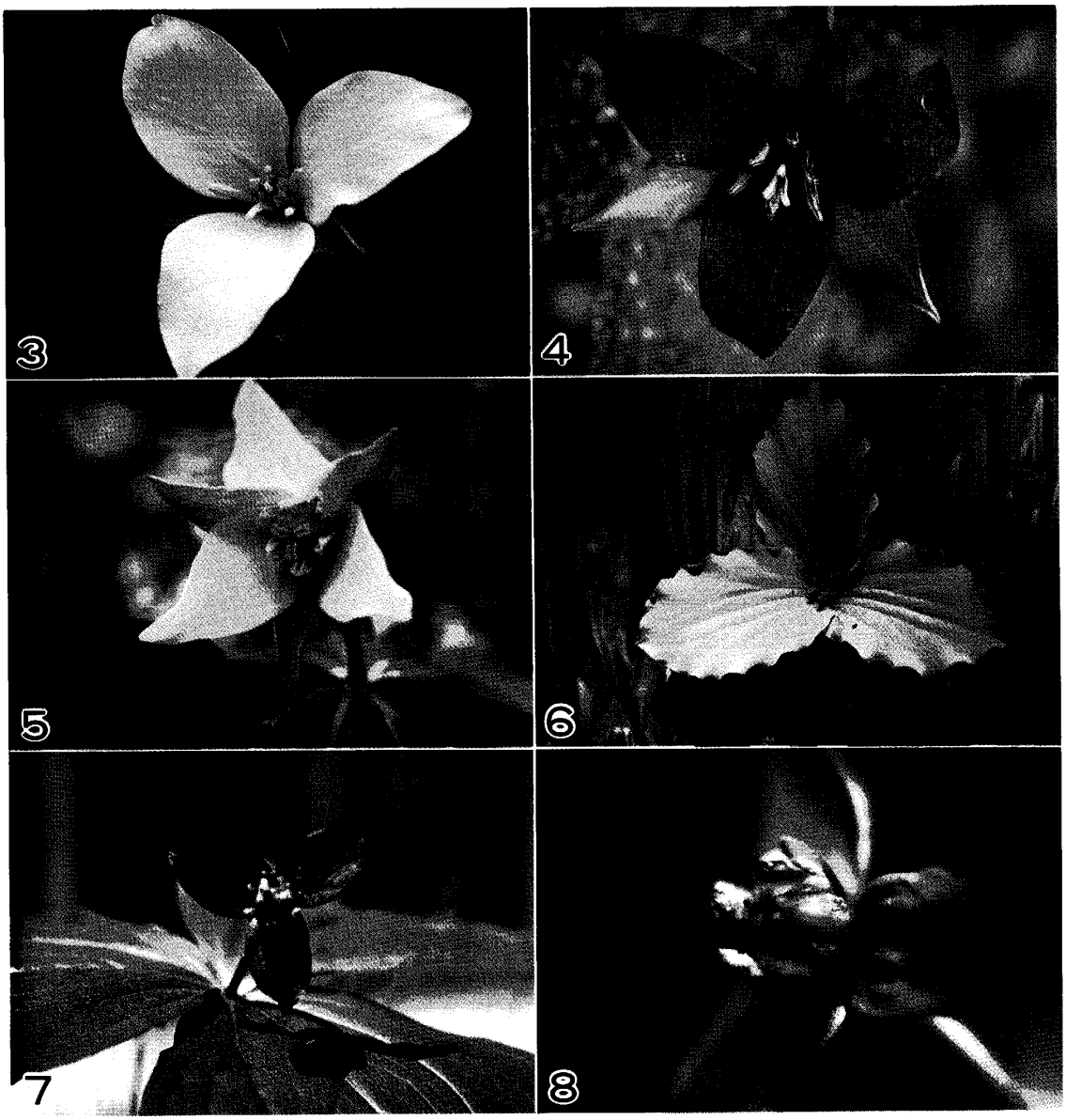


Fig. 2. Schematic figures of the same bands in Fig. 1. Black marks appear in all five species, but white circles are not universal.



Explanation of figures

Fig. 3. The flower of *T. kamtschaticum*.
Fig. 4. The flower of *T. erectum*.
Fig. 5. The flower of *T. tschonoskii*.
Fig. 6. The flower of *T. grandiflorum*.
Figs. 7 and 8. The flowers of *T. apetalon*.

Discussion

T. kamtschaticum (Fig. 3), *T. tschonoskii* (Fig. 5), and *T. apetalon* (Figs. 7 and 8) are representative species in eastern Asia. *T. erectum* (Fig. 4) and *T. grandiflorum* (Fig. 6) are representative species in North America. Of the above species, *T. erectum* has a red-purple flower, while *T. apetalon* has no petals but a red-purple color remains in the sepal (FUKUDA 1960; FUKUDA, MATSUMOTO and ICHINISE 1981). If we consider their relationship in terms of petal color, there is thus a similarity between *T. erectum* and *T. apetalon* as a red group, while *T. grandiflorum*, *T. kamtschaticum* and *T. tschonoskii* as a white group, are clearly different. However, our genetical results suggested a different conclusion.

As the present author has pointed out previously, the chromosome banding patterns of *T. kamtschaticum* are similar to those of *T. erectum*, but different from those of *T. grandiflorum* (FUKUDA 1990). *T. kamtschrticum* are numerous and include all the *T. erectum* patterns.

It is of great interest that the results of this electrophoretic analysis agreed with the chromosome banding information. That is, three the Asian species, *T. kamtschaticum*, *T. tschonoskii*, and *T. apetalon*, were correlated with the American *T. erectum*, but not with the American *T. grandiflorum*.

On the basis of this cytogenetical and molecular genetic evidences, a plausible hypothesis emerges. *T. erectum* might have migrated the Asian region and then establised *T. kamtschaticum*. In Asia, *T. kamtschaticum* is only a diploid species, while *T. tschnoskii* and *T. apetalon* are tetraploid species. *T. erectum* and *T. grandiflorum* are different genetically. *T. grandiflorum* could not have moved to the Asian region in the Tertiary period although the species have moved to the western North America (FUKUDA 1990). However, the *T. erectum* groups spread from the eastern North America to the northern Asia across the Bering Sea (there was a land in the Tertiary period) before the glaciations of the Pleistocene (FLINT 1957). *T. erectum* usually has red flowers, however, we sometimes see white flowered *T. erectum* as an albino type. Perhaps the *T. kamtschaticum* which arrived in Asia started with red flowered but turned immidiately white by means of a single gene. *T. apetalon* thus has a petalless flower, while the sepals retain the red color which might be originated from *T. erectum*. As the resuls, *T. erectum* might moved several times from the eastern North America to the easern North Asia. One of those has established *T. kamtschaticum*, the others have established T. *apetalon* and *T. tschonoskii* (Fig. 9). Further more studies of different isozymes by the

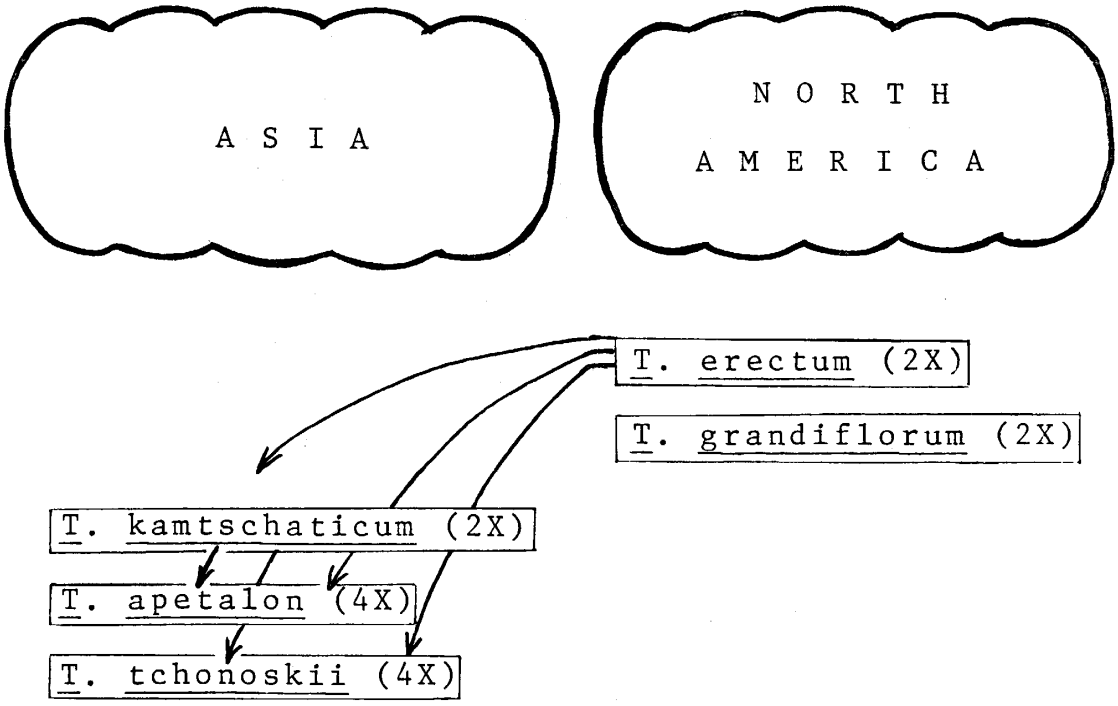


Fig. 9. Diagram showing schematically the possible evolutionary relationships among the Asian and North American *Trillium* species, as indicated by arrows.

electrophoresis will go on at the genetics laboratory of Tokyo Woman's Christian University.

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